Intérêt de l'anticorps Immunoglobulines G Anti Hsp70.1 dans le diagnostic de toxoplasmose oculaire

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Intérêt de l'anticorps Immunoglobulines G Anti Hsp70.1 dans le diagnostic de toxoplasmose oculaire

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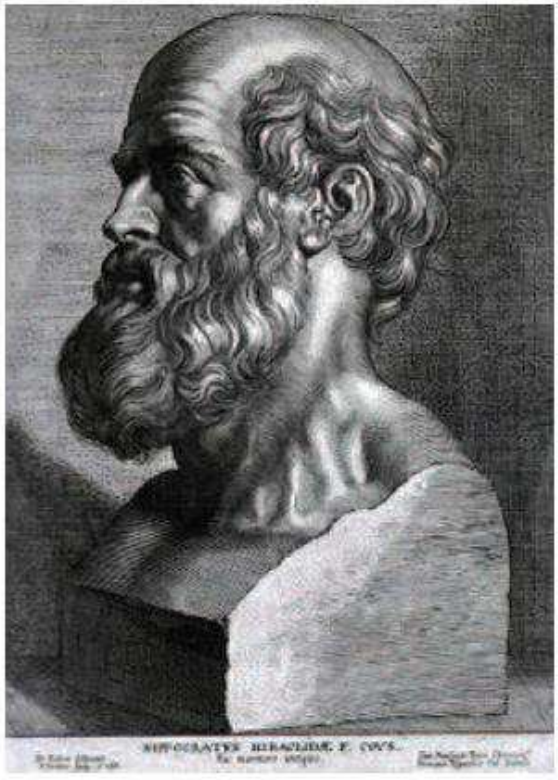
SERMENT D'HIPPOCRATE



En présence des Maîtres de cette Faculté, de mes chers condisciples et devant l'effigie d'Hippocrate,
Je promets et je jure d'être fidèle aux lois de l'honneur et de la probité dans l'exercice de la Médecine.
Je donnerai mes soins gratuitement à l'indigent et n'exigerai jamais un salaire au-dessus de mon travail. Je ne participerai à aucun partage clandestin d'honoraires.
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TABLE DES MATIÈRES

Prospective study of serum anti-Hsp70.1 IgG antibody levels in the diagnostic certainty of clinically suspected ocular toxoplasmosis

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Running head: Serum anti-Hsp70 antibodies in ocular toxoplasmosis

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Key words: ocular toxoplasmosis, uveitis, diagnosis, anti-Hsp70 antibody, *Toxoplasma gondii*

ABSTRACT

PURPOSE. Laboratory diagnosis of ocular toxoplasmosis (OT) needs to be improved mainly in clinical atypical cases. In a retrospective previous study, infected patients could display serum IgG anti-Hsp70.1 antibodies and that these antibodies could be used to improve the diagnosis of OT. The purpose of this multicentre prospective case-control study was to correlate clinical features with IgG anti-Hsp70 antibodies.

METHODS. We have included patients with confirmed (Group A1) or suspected OT (Group A2), and with cataract (Control Group B). The diagnosis was based on the ocular presentation, Goldmann-Witmer's coefficient, immunoblotting and/or PCR. Serum and aqueous humour (AH) were sampled at the time of uveitis. ELISA was used to measure serum anti-Hsp70.1-antibody levels. The OT patients were clinically followed up to six months and laboratory monitored up to three months.

RESULTS. Serum IgG anti-Hsp70.1 antibody levels were related to the affected retinal zone ($p=0.006$) and were correlated significantly to the retinal lesion size ($p<0.05$). Serum anti-Hsp70.1 and AH IgG anti-*Toxoplasma* antibodies were respectively positive in 10 and 5 out of 18 cases of Group A2 that were finally classified as true-clinically suspected OT.

CONCLUSIONS. Anti-Hsp70 may be involved directly or indirectly in the retinal lesions during ocular *Toxoplasma* infection and related to the affected retinal zone. Anti-Hsp70.1 antibody may confirm biologically suspected OT with an increasing specificity when associated with the presence of AH IgG anti-*Toxoplasma* antibodies. The value of AH anti-Hsp-70 antibody levels in the laboratory diagnosis of OT will further evaluated.

Toxoplasma gondii is a parasite widespread throughout the world; the seroprevalence has been estimated up to one third of the human population with considerable geographical differences that are also related to environmental, social and food hygiene factors.[1] Ocular toxoplasmosis (OT) may lead to a poor visual prognosis especially when the macula is impaired or when other complications occur.[2] Active creamy-white focal retinitis with or without a previous pigmented chorioretinal scar is the classical presentation of OT.[3-5] This presentation can also be atypical such as punctuate outer retinal toxoplasmosis, retinal vasculitis, retinal detachment, unilateral pigmentary retinopathy mimicking retinitis pigmentosa, neuroretinitis, papillitis and pseudo-multiple retinochoroiditis.[6, 7]

Laboratory testing is useful to confirm the infection in the cases of the atypical lesions, macular lesions and/or unexpected therapeutic response. For the diagnosis of OT, the sensitivity of the Desmonts' coefficient or Goldmann-Witmer coefficient (GWC) is 74% with a specificity of 100%, and these values may vary in terms of studies and when sampling was performed [8-10]. In atypical OT cases, GWC sensitivity may decrease to 36 % and specificity to 93% [11]. Despite improvement of diagnostic tests such as the Polymerase Chain Reaction (PCR) and quantification of anti-*Toxoplasma* antibodies [7, 12, 13], the sensitivity of the laboratory diagnosis remains unsatisfactory at the present time.

Heat shock protein 70 (Hsp70) is up-regulated during cellular stress, it is involved in the novo protein folding, refolding of stress denatured proteins, protein transport as well as in intracellular antigen processing and inflammatory process.[14-17] The expression of *T. gondii* Hsp70 and bag1/Hsp30 has been linked with bradyzoite growth and during the conversion of bradyzoites to tachyzoites during *Toxoplasma* reactivation.[18, 19] The expression of *T. gondii* Hsp70 is also related with the virulent

strains and is up regulated just before death of the host cell.[20, 21] Hsp70 is released from cells during necrosis and/or through physiological secretion mechanisms, enters the bloodstream and possesses the capacity to operate at distant sites in the body.[22] Moreover, significant protection against *Toxoplasma* infection in B6 and Balb/C mice may be induced by vaccination with *T. gondii* Hsp70 gene that is associated with dentritic cell activation and Th1 polarization.[23, 24] Anti-Hsp70 IgG autoantibody, produced by B-1 cells of C57BL/6 mice, has been observed after *T. gondii* infection.[25, 26]

The presence of human anti-Hsp70.1 antibody has been previously investigated in a retrospective study with patients clinically suspected of OT.[27] We showed: a) the serum anti-Hsp70.1 antibody test could confirm clinically suspected OT and b) these levels correlated to the retinal lesion size.[27] We hypothesized that anti-Hsp70 antibodies: (1) are also present in aqueous humour of clinically suspected OT patients and their quantifications may improve the diagnostic strategy; (2) decrease in the follow-up of OT patients, and (3) are not present in patients with cataract.

The purpose of this prospective multicentre study was to confirm the contribution of anti-Hsp70 IgG antibody in the diagnosis of OT and their relationship with the retinal lesion size. The secondary objectives were the study: (1) of the association among anti-Hsp70 IgG antibody levels and clinical characteristics of inflammation, and (2) the contribution of anti-Hsp70 IgG antibody levels during the clinical follow up.

PATIENTS, MATERIALS AND METHODS

Patient and control groups

Our research adhered to the tenets of the Declaration of Helsinki and to Good Clinical Practice guidelines and was approved by the local Institutional Review Board (IRB #6705). This multicentre prospective study included patients from four French hospitals (Grenoble, Chambery, Saint-Etienne and Valence) between 2010 and 2013. The inclusion criteria were patients with uveitis and one or more retinitis lesions (Group A1, confirmed OT and Group A2, suspected OT) or patient operated on for cataract surgery (control Group B) being older than 18 years old and signed informed consent. The exclusion criteria were: pregnant woman, nursing mother, patients refusing anterior chamber puncture and patients with non-predictable follow-up. Group A2 was divided in two sub-groups: true suspected clinically OT (Sub-group A2-Toxoplasmosis (A2-T)) and other uveitis different than OT (Sub-group A2-Non-Toxoplasmosis (A2-NT)). The Gold standard group was composed of Group A1 and Sub-group A2-T.

The definition of clinical OT was the presence of at least one active creamy-white focal retinal lesion in either eye combined or not with pigmented lesion scars in either eye[3-5]. Atypical clinical OT was defined as neuroretinitis, papillitis, iridocyclitis, retinal detachment and acute retinal necrosis[6].

GWC is defined as X/Y; where X = *Toxoplasma* IgG antibody in aqueous humour (AH) divided by total IgG in AH; and Y = *Toxoplasma* IgG antibody in serum divided by total IgG in serum. Laboratory confirmed OT required at least one of the following criteria: a GWC ≥2; AH anti-*T. gondii* neo-synthesized antibodies detected by immunoblotting (WB, *Toxoplasma* Western-blot IgG-IgM, LDBIO Diagnostics, Lyon, France); and positive genomic *T. gondii* DNA amplification by polymerase chain

reaction (PCR) in the AH (target *T. gondii* rep452, LightCycler 2.0, Roche, Grenoble, France).[9, 12, 28, 29 30]

For each patient, five visits with a detailed ocular examination were performed: at time of inclusion, at 3, 6, 12 and 24 weeks. The data were collected at each site using the same case report form (CRF). The OT patients were laboratory monitored up to 12 weeks. Underlying diseases, immunosuppression and other causes of uveitis were also examined.

Patients were grouped according to clinical and biological criteria. Group A1 included patients with at least one manifestation of retinitochoroiditis and laboratory confirmed OT by at least one three tests cited above. Group A2 included patients with at least one manifestation of retinitochoroiditis and non-laboratory confirmed OT by any of the three tests cited above. Control Group (B) included patients with cataract without associated ocular disease (including uveitis or diabetes).

Ophthalmologic parameters

Each patient underwent a completed ophthalmologic examination, including the best corrected visual acuity (BCVA) expressed in logarithm of minimum angle of resolution (LogMAR), intraocular pressure (IOP), score of inflammation (anterior chamber cells and the reflection of light from proteins in the AH (flare)),[31] vitritis, number and localization of active lesions and scar related to posterior and anterior segments,[32] retinal vasculitis, retinal necrosis, or papillitis.[33]

Colour retinal fundus (Topcon TRC50EX®, Tokyo, Japan) was performed at each visit. Fluorescein and indocyanine green (ICG) angiographic photographs (Spectralis ® HRA-OCT, Heidelberg, Germany) were also performed at the inclusion visit. Images were analysed by counting pixels using the Image J software (National

Institute of Health) for the calculation of the ratio of the lesion size and size of the optic nerve disc on ICG and colour frames. However good quality photos could not be done for all patients caused by cataract or vitritis.

A mapping of the posterior pole was performed by optical coherence tomography (OCT) (Spectralis®, HRA-OCT, Heidelberg, Germany). Foveal central subfield thickness was defined as the average thickness in the central 1-□m-diameter circle of the Early Treatment Diabetic Retinopathy Study (ETDRS) region after verification of the centring the macula.

Location of lesion was defined within zone 1 of the retina (region extending 3000 µm from the foveal centre), zone 2 (the region extending from the border of zone 1 to the equator) and zone 3 (the region from the border of zone 2 to the ora serrata).[34]

Anti-*Toxoplasma* therapy

In cases of active toxoplasmic retinochoroiditis, the anti-*Toxoplasma* treatment was at the discretion of the investigator: pyrimethamine (1 mg/kg/day; n=9), sulfadiazine (100 mg/kg/day) and folinic acid (50 mg/week) or trimetroprim (160 mg/day; n=24) + sulfamethoxazole (800 mg/day) (TMP/SMX) or pyrimethamine (1 mg/kg/day; n=15) + azithromycin (500 mg/day) for at least six weeks. The initial anti-*Toxoplasma* treatment was changed if adverse effects were noted. The anti-*Toxoplasma* treatment was prolonged in OT cases if healing of ocular lesion was not observed.

Combination of anti-parasite drugs with corticosteroids (prednisone per os at initial dosage of 1 mg/kg/day) was usually administered and tapered for limited periods of 1 or 2 months in case of active toxoplasmic lesions near the macula or optic nerve or in case of severe intra-ocular inflammatory response.[35]

Anti-*Toxoplasma* antibodies

Indirect Immunofluorescence and Enzyme Linked Fluorescent Assay (ELFA) screened all groups for serum IgG and IgM anti-*Toxoplasma* antibodies (Toxo IgG, IgM, bioMérieux, Marcy l'Etoile, France)[36]. The combination of ISAGA (Toxo IgM, bioMérieux) and IgG avidity test (VIDAS, bioMérieux) was used to confirm acute toxoplasmosis in patients.[37]

Anti-Hsp70.1 antibodies

Serum anti-Hsp70.1 levels were assayed by ELISA as described elsewhere [27, 38] but with some modifications: a) coating was with 100 µl of human recombinant Hsp70 (NSP-555, Stressgen- Enzo Laboratories, Villeurbanne, France) /well at 2 µg of protein/ml; b) each assay was repeated three times; c) the wells were incubated with 100 µl of sera diluted to 1/200 for 1 h at 37°C; d) the enzymatic activity was developed with 0.75 mg/ml *o*-phenylene-diamine dihydrochloride, 0.03 % sodium perborate in 0.05 M citrate phosphate buffer, pH 5, for ten minutes at room temperature in the dark. The reaction was stopped with 200 µl of 2 N H_2S0_4 and absorbance read at 492 nm. Reproducibility for intra and inter assays was higher than 95%. The confirmation of the test specificity was established by inhibition of the antibody response with human recombinant Hsp70 (5µg/ml).[38]

Determination of cut-offs

The most appropriate cut-off level of IgG anti-Hsp70.1 antibodies was determined as previously described except for using the cataract Group B without uveitis and diabetes as control instead the blood-donor healthy group[38]. Briefly, the cut-off was selected by the best addition of the Youden's index and Yule's Q coefficient. The cut-offs were represented and calculated in a receiver operating characteristic (ROC) curves for serum IgG anti-Hsp70.1, and AH IgG anti-*Toxoplasma* antibodies.

Statistical analysis

For data with normal distribution the mean, standard deviation (SD) and the range were given, otherwise the median, 10^{th} centile and 90^{th} centile were reported. Non-parametric tests were employed. The correlations were established with Z correlation coefficients. Kruskal-Wallis Test (3 groups) and then by the Mann-Whitney's U Test (2 groups) were used to establish significant differences between data sets. Friedman's test (3 groups) and then Wilcoxon's rank test (2 groups) were used for paired variables. The statistical analysis was performed using Statview software (SAS Institute Inc.). Statistical significance was defined as a two-tailed p-value less than 0.05.

RESULTS

The characteristics of the enrolled patients are shown in Table 1. Twenty-one patients were included with clinically suspected and biological confirmed OT (Group A1), 30 patients with a clinically suspected OT but lacking laboratory confirmation (Group A2) and 42 patients with cataract (Control group B, Table 1). The biological confirmation was done by GWC and/or PCR and/or immunoblotting (Table 1). The number of retinochoroiditis-affected eyes was 24 and 32 for Group A1 and A2, respectively (Table 1). At the beginning of the study, eleven of these affected eyes (5 in Group A1 and 6 in Group A2) presented bordered pigmented and active lesions indicating a possible *Toxoplasma* reactivation with or without a previous OT history. Ten patients (6 in Group A1 and 4 in Group A2) presented medical history of former OT episodes. None of these ten patients had a medical history of congenital toxoplasmosis. Patients' BCVA gain was $\geq$ 2.8 lines at 3 months (Table 1). Retinal lesion size, IOP, and mean time of ocular lesion healing are also shown in Table 1 as well as the toxoplasmic serological status of the patients.

Serum IgG or IgM anti-*T. gondii* antibody levels in the three study groups (Figure 1)

The three groups A1, A2 and B showed a significant statistical difference in the levels of serum IgG or IgM anti-*Toxoplasma* antibodies (p=0.009 and p=0.02, respectively; figure 1). Significant differences were observed between A1 and B groups for IgG (p=0.02) and IgM anti-*Toxoplasma* antibodies (p=0.004) as well as for IgG anti-*Toxoplasma* antibodies (p=0.008) between A2 and B groups. Non-statistical significant differences in the levels of serum IgG or IgM anti-*Toxoplasma* antibodies were observed between A1 and A2 groups.

Serum anti-Hsp70.1 IgG antibodies among the groups

The levels of serum IgG anti-Hsp70.1 antibodies among the three groups at the time of inclusion are shown in Figure 2a. Figure 2b shows the levels of anti-Hsp70.1 antibodies in the sub-group A2-T (toxoplasmosis), true suspected clinically OT, and A2-NT (non-toxoplasmosis), other uveitis different than OT. The kinetics of serum IgG anti-Hsp70.1 antibody levels in three patients is illustrated in Figure 3a-c. The Figure 3d shows the box plots of serum IgG anti-Hsp70 antibody levels in the gold standard group during the follow up at to 24 weeks. These levels had a tendency to decrease during the follow-up.

Relationships among OT parameters, local and systemic immunity

Table 2 shows the relationships among OT findings, ocular and systemic immunity. Serum IgG anti-Hsp70.1 antibodies were significantly correlated with the chorioretinal lesion size. Despite lesion size measured by ICG was higher than size obtained by the colour photographs (n=27, p=0.0001) both measurements were highly correlated (r=0.919, p<0.0001).

Serum IgG anti-Hsp70.1 antibodies were related to the affected retinal zones (n=41, p=0.006; Figure 4) with higher values in affected retinal zone 1 (0.361 ±0.218) and 2 (0.341 ±0.186) (n=30, p=0.002 and n=17, p=0.005, respectively) than in the zone 3 (0.151 ±0.040). After the Bonferroni adjustment the p values reminded significantly.

AH IgG anti-*Toxoplasma* antibody levels were not statistically related to the affected retinal zones (n=29, p=0.23): zone 1 (9.2 (mean) ± 20.1 (SD)), zone 2 (20.2 ±27.9) and zone 3 (3.4 ±4.7).

Serum IgG anti- *Toxoplasma* antibody levels were not statistically associated to the affected retinal zones (n=41, p=0.992): zone 1 (212 ±386); zone 2 (258 ±533) and zone 3 (304 ±488).

Serum IgM anti- *Toxoplasma* antibody levels were equally not statistically connected to the affected retinal zones (n=41, p=0.136): zone 1 (1.07 ±2.14); zone 2 (0.43 ±1.06) and zone 3 (0.08 ±0.08).

The affected retinal zones were related to the number of active chorioretinal lesions (n=39, p=0.04): zone 1 (1.3 ±0.8); zone 2 (0.9 ±0.3) and zone 3 (1.5 ±0.5). The number of active chorioretinal lesions was significantly higher in affected retinal: zone 1 than zone 2 (n=33, p=0.05) and zone 3 than zone 2 (n=16, p=0.02).

Serum IgG anti-Hsp70.1 antibody levels were not associated to the presence (0.349 ± 0.237) or absence (0.308 ±0.182) of macular oedema (n=44, p=0.94) nor with the presence (0.258 ±0.065) or absence (0.328 ±0.185) of vasculitis at the time of patient's inclusion in the study (n=40, p=0.52).

Serum IgG anti-Hsp70.1 antibody levels were not related with the three groups of vitritis formed in terms of vitreous haze grading scale: A <1; B ≥1 and ≤2; C >2: (n=44, p=0.11).

Discrimination among groups using serum IgG antibodies anti-Hsp70 and AH IgG antibodies anti-*Toxoplasma*

Table 3 shows the evaluation of diagnostic value of OT using the serum IgG antibodies anti-Hsp70 and AH IgG antibodies anti-*Toxoplasma* in terms of the cut-offs obtained by the ROC curve. Ten and five patients of sub-Group A2-T were positive in serum IgG anti-Hsp70 and AH IgG anti-*Toxoplasma* antibodies, respectively.

After revision of clinical data, a gold standard Group was formed with Group A1 and Sub-Group A2-T (Figure 5a). The Sub-Group A2-T was a part of the Group A2 and represents the true clinically OT cases (Figure 5a). Ten out of 39 patients of the gold standard group (25.6%) presented medical history of OT episodes from one to 46 months before the study. Three patients presented OT recurrences from one to six months during the study and two patients an OT recurrence after 18 months.

Serum IgG anti-Hsp70 antibodies were not statistically different among the OT gold standard, non-OT and control groups (n=93, p=0.57).

AH IgG anti-*Toxoplasma* antibodies were statistically different among confirmed OT, true clinically OT and other uveitis different than OT (n=32, p=0.002). These antibodies were significantly higher between confirmed OT (A1) than in true clinically OT (A2-T; n=25, p=0.01) and between A1 and other uveitis different than OT (A2-NT; n=21, p=0.002). However no significant difference was observed between A2-T and A2-NT sub-group (n=18, p=0.30).

Value of serum anti-Hsp70.1 antibodies to confirm clinically suspected cases of OT

The number of biologically confirmed OT cases was 21 (Group A1) after taking into account the sensitivities of the tests used. After review of the clinical records of Group A2 and the evolution after treatment, the OT clinical diagnosis was considered for 18 (A2-T Sub-Group) out of 30 patients (Figure 5a); Ten out of these 18 patients

were found positive for serum IgG antibodies anti-Hsp70. Four out of these ten patients (nd=3) were positive for AH IgG anti-*Toxoplasma* antibodies. We have established that four out of the twelve patients (A2-NT Sub-Group) who had initially uncertain diagnosis or not diagnosed as OT, were positive for serum IgG anti-Hsp70 antibodies while other diagnosis were finally selected: idiopathic occlusive vasculitis (n=1), endogenous endophthalmitis (n=1), PCR-proved-HSV keratouveitis (n=1) and idiopathic uveitis (n=1). Absence of AH IgG anti-*Toxoplasma* antibody was observed in these four cases. Thus, AH IgG anti-*Toxoplasma* antibody test can help to increase the specificity of the anti-Hsp70 serology. A case of VZV acute retinal necrosis presented positive AH IgG anti-*Toxoplasma* antibody levels were associated with negative anti-Hsp70 serology. In the eight patients of the A2-NT Sub-Group with negative IgG anti-Hsp70 serology, the other diagnoses were: PCR-proved-HSV keratouveitis (n=3), idiopathic uveitis (n=1), multifocal chorioretinitis (n=1) and PCR-proved-VZV acute retinal necrosis (n=3).

DISCUSSION

Our results of this prospective study show the presence of anti-Hsp70.1 antibodies in sera of patients with laboratory confirmed and clinically suspected OT that were related to the affected retinal zone and to retinal lesion size. These findings are consistent with the production of anti-Hsp-70 auto-antibodies by resistant Balb/C and susceptible C57BL/6 mice [25] and with our previous retrospective report.[27] The conserved Hsp70 N-terminal ATP binding domain of *T. gondii* may be involved in the induction of IgG antibodies anti-Hsp70 by a cross reaction mechanism.[26, 39].The production of these antibodies is transitory in mice and in all probability in humans as demonstrated in several cases in the present study.[40 27] Antibody transfer across the

blood retina barrier is uncommon [41]. The presumably hypothesis of Chen et al was that *T. gondii* Hsp70 could penetrate the blood-retina barrier.[25] Calderwood et al suggested that Hsp70 can go into the bloodstream after release of intracellular Hsp70 by active secretion mechanisms or following necrosis.[22] *T. gondii* Hsp70 might be captured by antigen presenting cells via their Hsp70 receptors and be carried through the trabecular meshwork via the blood vessels.[42] A relationship between intraocular toxoplasmic immunity and the systemic immunity is suggested by a positive correlation between these antibodies and the retinal lesion size in the present prospective study and in the former study.[27]

We consider that the gold standard for the diagnosis of OT remains the ophthalmologic examination, although atypical lesions may complicate the clinical diagnosis. [6, 7, 43, 44] Laboratory tests are then usually used to confirm the suspicion of OT. In the present prospective study, fourteen of our cases were confirmed by a GWC≥2, four by PCR, and five by immunoblotting. Additional diagnosis was suggested in ten and five patients of sub-Group A2-T by the presence of serum IgG anti-Hsp70 and AH IgG anti-*Toxoplasma* antibodies, respectively. As observed in the present and in the former study anti-Hsp70 antibody responses are in some cases complementary to the classical immunological response to *T. gondii* antigens.[27] This complementary immune response may be due to the deviant immune response in the eye. [45, 46] The fact that the eye immune response comprises the classical and non-classical ones with some particularities was supported elsewhere. [47] To confirm clinically suspected OT cases, we recommend the following algorithm: a) perform GWC and/or PCR and/or immunoblotting; b) if GWC or PCR or immunoblotting are not informative carry out complementary analysis with the anti-Hsp70 antibody test; c) if this test is positive the clinically suspected case could be confirmed. Nevertheless,

Hsp70 may be expressed as a result of other diseases like ocular myasthenia gravis and thus the ophthalmologic information should always be taking into reflection when diagnosing OT.[48] In the present study the specificity of the anti-Hsp70 test was increased when associated with the presence of AH IgG anti-*Toxoplasma* antibodies. This also emphasizes the significance of the kinetics of evolution of anti-Hsp70 antibody levels in each patient, before, during and after the episode of OT and of obtaining the ophthalmological clinical data. After the successful treatment of OT, serum anti-Hsp70 may decrease as do the retinal lesion size; these results emphasize the possible relationship between levels of serum anti-Hsp70 antibodies and the lesion size. In immunocompetent OT patients, serum anti-Hsp70 antibodies may be regarded as a potential marker of OT. This conclusion is in agreement with the fact that a) Hsp70 is expressed during *T. gondii* growth and differentiation [18-20]; b) Hsp70 expression is related to the virulence strain [49]; c) Anti-Hsp70 IgG antibody has been observed experimentally in mice after *T. gondii* infection [25, 26] and in humans during OT episodes.[27]

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No conflicts of interest exist for any of the authors.

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FIGURE LEGENDS

FIGURE 1. Box graph of serum IgG and IgM anti-*T. gondii* antibodies in the control group (B) and in suspected toxoplasmic uveitis patients (A1 and A2).

IgG (**a**), IgM (**b**) antibody anti- *T. gondii* levels observed by ELFA in sera of confirmed OT (A1), clinically suspected OT (A2) and control (B) patients. All data represents one sample per patient. The box-and-whisker plots show: the median of 21 (A1), 30 (A2) and 42 (B) measurements, respectively; the lower and upper quartiles (box) and 10^{th} to 90^{th} centiles (vertical bars); points above or below these levels are shown separately. The P-values for data from figure 1A and 1B were 0.009 and 0.02, respectively. Significant differences were observed between Group A1 and B (for IgG and IgM) as well as between Group A2 and B (only for IgG).

FIGURE 2. Serum levels of anti-Hsp70.1 antibodies in the control Group (B) and in suspected toxoplasmic uveitis patients (A1 and A2).

(**A**) The box-and-whisker plots show: the median of 21 (A1), 30 (A2) and 42 (B) averages of 3 measurements, respectively. (**B**) The box-and-whisker plots show: the median of 18 (A2-T) and 12 (A2-NT) averages of 3 measurements, respectively; the lower and upper quartiles (box) and 10^{th} to 90^{th} centiles (vertical bars); points above or below these levels are shown separately. All data represents one sample per patient.

FIGURE 3. Kinetics of serum IgG anti-Hsp70.1 antibodies in patients suspected of OT.

During this study the ocular lesions were healed in an average of 13.3±6.9 (from 5.1 to 31 weeks) after the first clinical sign of ocular inflammation. The patients were laboratory followed-up to 3 months. A sample collected at the inclusion visit was considered as the patient's reference signal. A) In one patient with laboratory

confirmed OT (Group A1) a significant decrease (47.4%) in the marker was observed (from Day 20 to Day 82); B) In one patient classified as having true-clinically OT (Sub-Group A2-T) a significant decrease (64.2.%) in the marker was observed (from D5 to D87); C) In one patient with other ocular inflammation different than OT (Sub-Group A2-NT) in who a significant decrease of the marker was not observed and (D) Levels of serum IgG anti-Hsp70 antibodies during the study in the OT gold standard group. The box-and-whisker plots of Figure 3D show: the median at 0, 6 and 24 weeks, respectively; the lower and upper quartiles (box) and 10^{th} to 90^{th} centiles (vertical bars); points above or below these levels are shown separately. The medians had a tendency to decrease during the follow-up.

FIGURE 4. Box graph of serum IgG anti-Hsp70.1 antibodies according to the location of the chorioretinal lesion.

Serum IgG anti-Hsp70.1 antibodies were different according to the location of the chorioretinal lesion (n=41, p=0.006). Serum IgG anti-Hsp70.1 antibody levels were higher in affected retinal zone 1 (n=30, p=0.002) and 2 (n=17, p=0.005) than in the corresponding zone 3.

FIGURE 5. Serum IgG anti-Hsp70.1 antibody levels in the Gold standard group of toxoplasmic uveitis.

(**A**) Flow chart describing the OT gold standard group. (**B**). Figure 5B illustrates the variation of the serum IgG antibodies anti-Hsp70 in terms of the OT gold standard, Sub-Group-A2-NT and control Group B.

TABLE 1. Characteristics of the patients of the study

Parameters	Group A1	Group A2	Group B
Ocular toxoplasmosis	Confirmed	Suspected	Absent
Number of patients	21	30	42
Mean age years ± SD*	41.1 ±15.5	51.6 ±21.2	73.6 ±13.9
Male/Female	15/6	19/11	21/21
Retinochoroiditis: affected eye	24	32	0
Lesion size, disc diameter (range)	1.9± 1.4 (0.5-6)	1.1± 0.9 (0-4)	
BCVA* (LogMAR) t0 ± SD	0.73 ±0.80	0.59 ±0.77	0.65 ±1.08
BCVA (LogMAR) t3months ± SD	0.45 ±0.63	0.29 ±0.52	
Improvement BCVA* (LogMAR)	0.28	0.30	
Mean time of healing (weeks) ± SD	13.6 ±5.8	13.1 ±7.6	
Goldmann-Witmer Coefficient, GWC	Positive (n=14/16)	Negative (n=9/9); Incalculable (n=8/8)†	ND*
DNA *T. gondii* PCR	Positive (n=3/6)	Negative (n=14/21)	ND
Immunoblotting (WB)	Positive (n=5/6)	Negative (n=6/6)	ND
Aqueous humour (AH) IgG anti-*Toxoplasma*: IU/ml, median (range)	11 (0.9-83)	0 (0-18)	ND
Acute/Acquired toxoplasmosis ‡	2/19	3/26	0/42

Note: *BCVA: best correct visual acuity; IU: international unit; ND: not done; SD: standard deviation; † absence of AH IgG anti-*Toxoplasma* antibodies; ‡ ISAGA, avidity test.

TABLE 2. Z correlation coefficient (Z) among AH IgG anti-*Toxoplasma* antibody, chorioretinal lesion size, number of lesion, IOP, healing of lesion, central macular thickness by optical coherence tomography (OCT), and serum IgG anti-Hsp70.1 antibodies

Serum IgG anti-Hsp70.1 antibody and	**Z**	**P**
AH IgG anti-*Toxoplasma* antibody (n=32)	0.263	0.15
Lesion size by colour image (DD; n=32)	0.381	0.03
Lesion size by ICGA (DD; n=30)	0.343	0.06
Number of lesion (n=42)	-0.064	0.69
Intra ocular pressure (n=43)	-0.184	0.24
Healing of lesion in weeks (n=38)	0.077	0.65
Central macular thickness, □m (n=29)	0.249	0.20

NOTE. DD: optical disc diameter; ICGA: indocyanine green angiography; Z: Z correlation coefficient.

TABLE 3. Discrimination among groups with suspected clinically OT (A1 and A2) and control Group B using serum IgG anti-Hsp70.1 and AH IgG anti-*Toxoplasma*

Parameter	**IgG antibody anti-**	
	Hsp70.1 (serum)	***Toxoplasma* (AH)**
Youden's index	0.191	0.607
Yule's Q coefficient	0.386	0.895
Cut-offs	0.284	0.9 IU/ml
Area under de curve (AUC)	0.58	0.79
Group A1; (n=21) % (95 % CI) [†]	43 (9/21)[†] (24-64)	93 (13/14)[†] (72-100)
Group A2 (n=30) % (95 % CI)	47 (14/30)[†] (30-64)	32 (6/19)[†] (15-54)
Sub-Group A2-T (n=18) (95 % CI)	56 (10/18)[†] (34-76)	45 (5/11)[†] (21-72)
Sub-Group A2-NT (n=12) (95 % CI)	33 (4/12)[†] (13-60)	14 (1/7)[†] (11-48)
Cataract Control Group B (n=42) % (95 % CI)	24 (10/42)[†] (14-37)	Not done
Sensitivity (%) confirmed clinical OT	49 (19/39)	75 (18/24)
Specificity (%)	70	85.7
Positive Predictive Value (%)	54	94.7
Negative Predictive Value (%)	66	50

NOTE. n= number of patients; AH= Aqueous humour. OT= Ocular toxoplasmosis

[†] Prevalence in %; between first parentheses number of positive cases out of number of total cases; and second ones 95% confidence intervals (95% CI).

FIGURE 1. Box graph of serum IgG and IgM anti-*T. gondii* antibodies in the control group (B) and in suspected toxoplasmic uveitis patients (A1 and A2).

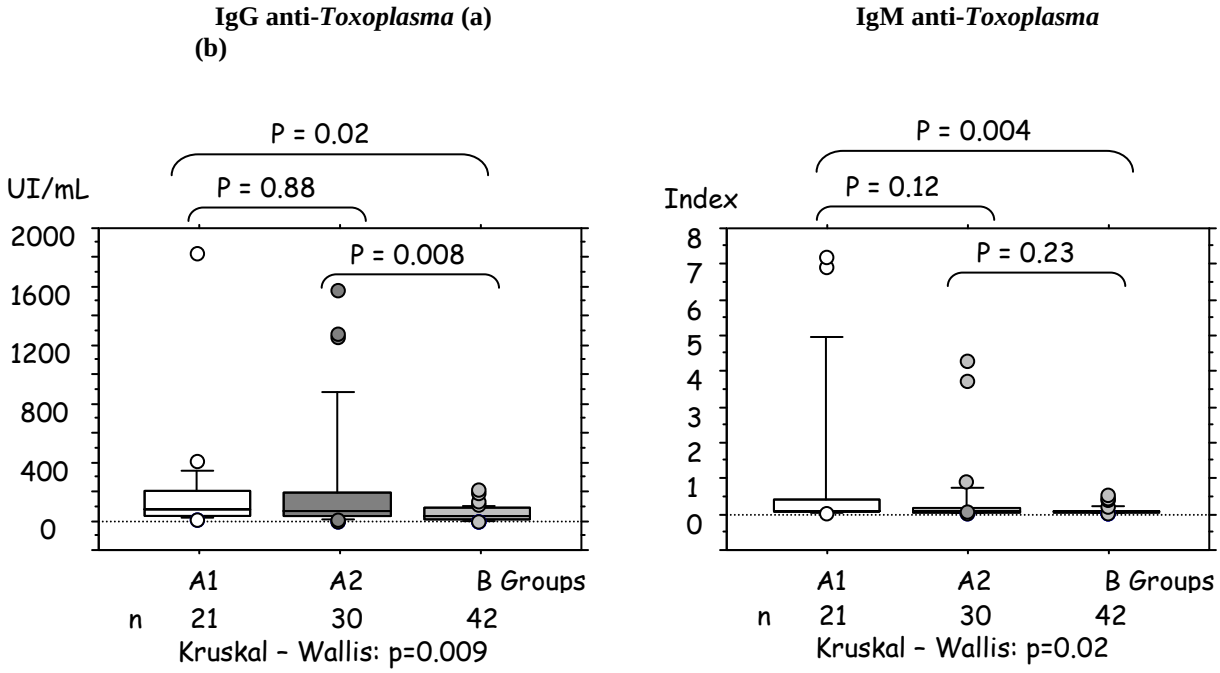


FIGURE 2. Serum levels of anti-Hsp70.1 antibodies in the control Group (B) and in suspected toxoplasmic uveitis patients (A1 and A2).

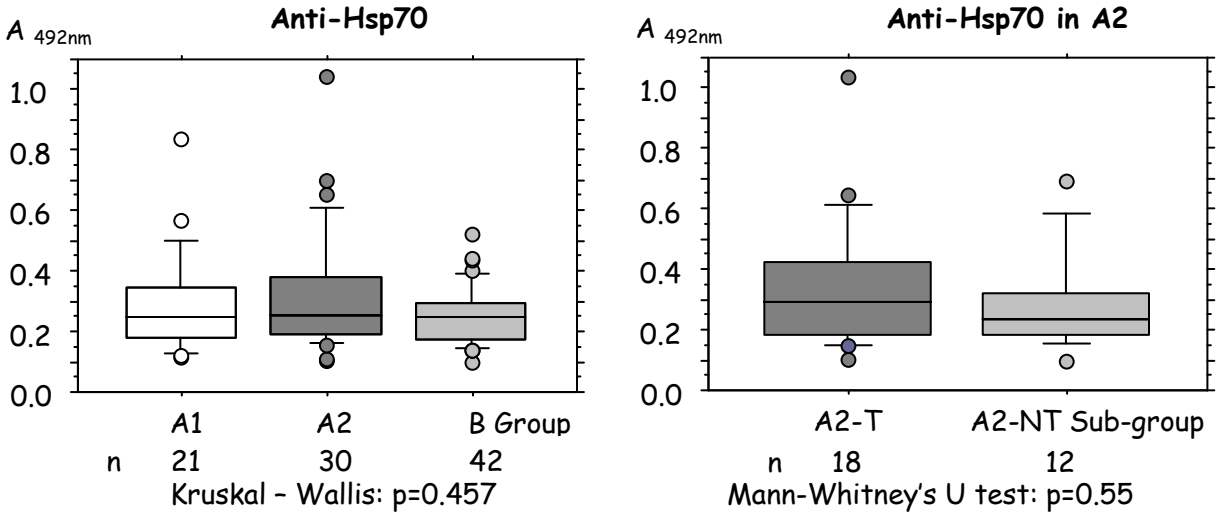
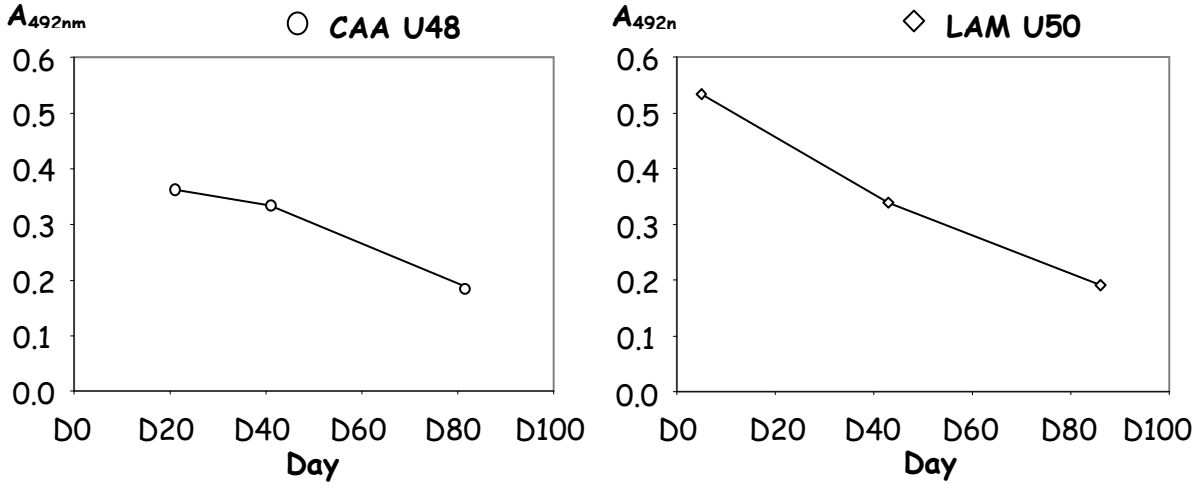


FIGURE 3. Kinetics of serum IgG anti-Hsp70.1 antibodies in patients suspected of OT.

A: Confirmed OT (A1) **B: Confirmed Clinical OT (A2-T)**



C: Non Toxoplasmic (A2-NT) uveitis **D: Gold Standard Group**

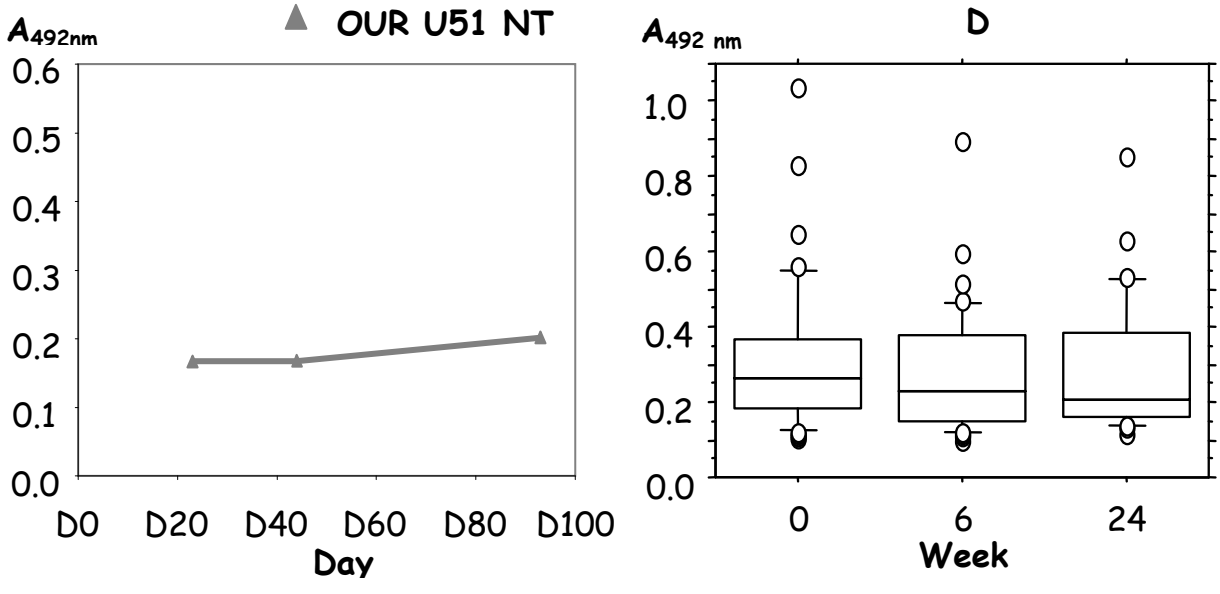


FIGURE 4 Box graph of serum IgG anti-Hsp70.1 antibodies according to the location of the chorioretinal lesion.

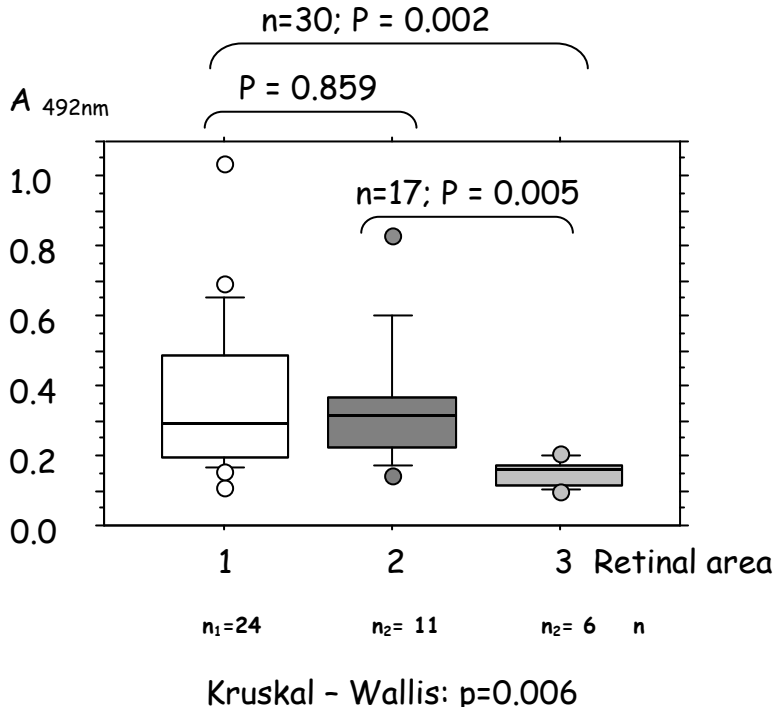
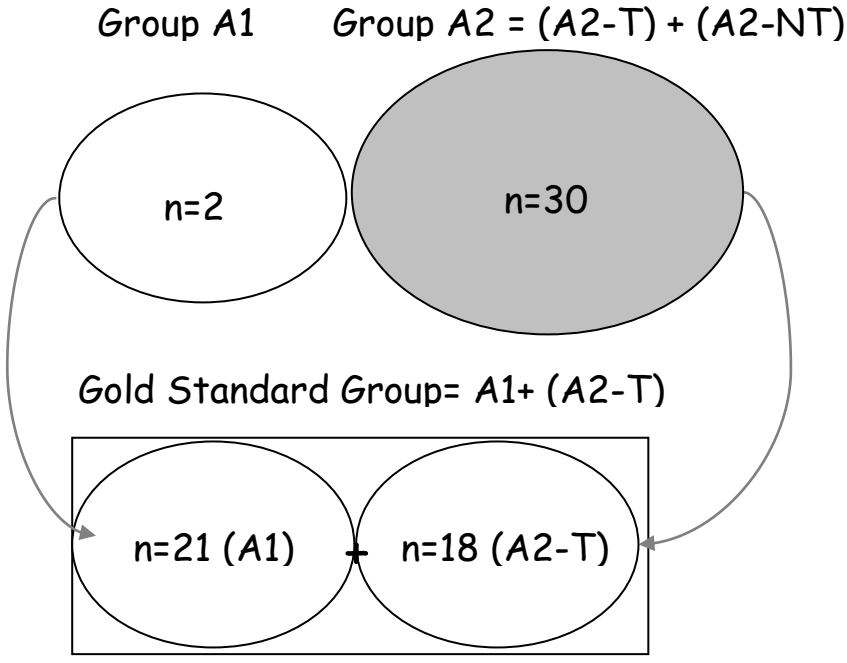
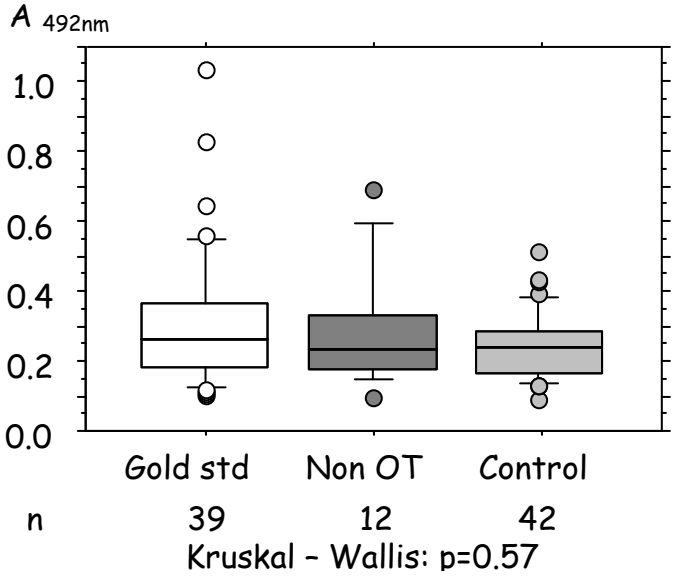


FIGURE 5. Serum IgG anti-Hsp70.1 antibody levels in the Gold standard group of toxoplasmic uveitis.

(A) Flow chart describing the OT gold standard group



(**B**). Box graph of serum IgG antibody anti-Hsp70 in OT Gold standard, Non-OT and Control group



THESE SOUTENUE PAR : Antoine LESOIN

TITRE : Intérêt de l'anticorps Immunoglobulines G Anti Hsp70.1 dans le diagnostic de toxoplasmose oculaire

La toxoplasmose oculaire est la première cause d'uvéite postérieure, dont le retentissement peut conduire à la cécité. L'objectif de cette étude était d'améliorer le diagnostic des formes atypiques de toxoplasmose oculaire (TO) grâce au dosage sérique d'un nouveau marqueur l'anticorps Immunoglobulines G Anti Hsp70.1 (Ac IgG Anti Hsp70.1).

Cette étude prospective et multicentrique a inclus 21 patients atteints de TO confirmés par la biologie, 30 patients suspects de toxoplasmose présentant une choriorétinite et 42 patients témoins (atteints de cataracte). La confirmation biologique faisait appel au coefficient de Goldmann-Witmer, Western Blot ou la PCR. Les taux d'anticorps ont été déterminés par méthode ELISA.

La valeur sérique de l'Ac IgG anti Hsp70.1 était significativement différente selon la zone de rétine atteinte (p=0.006) ainsi qu'en fonction de la taille de la lésion choriorétinienne (p=0.03). La détermination par la méthode de la courbe ROC d'un seuil de l'Ac IgG anti Hsp70.1 à partir des valeurs des Anticorps anti toxoplasmose dans l'humeur aqueuse n'a pas montré de différence entre nos 3 groupes (p=0.57).
Dans le groupe suspect de toxoplasmose, la mesure de l'Ac IgG anti Hsp70.1 a permis de confirmer biologiquement 10 patients sur 18 présentant une TO clinique.

En conclusion, l'Ac IgG Anti Hsp 70.1 peut être utilisé, en complément des autres méthodes biologiques, pour confirmer l'étiologie toxoplasmique d'une choriorétinite. Une étude complémentaire de son dosage dans l'humeur aqueuse devrait permettre de mieux appréhender la relation entre l'Ac IgG Anti Hsp 70.1 et le diagnostic, ainsi que la sévérité de la toxoplasmose oculaire

VU ET PERMIS D'IMPRIMER
Grenoble, le 10/10/2013

LE DOYEN
M. le Professeur J.P. ROMANET

LE PRESIDENT DE LA THESE
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